Host immune response to *Toxoplasma gondii* and *Ascaris lumbricoides* in a highly endemic area: evidence of parasite co-immunomodulation properties influencing the outcome of both infections

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*Toxoplasmosis and ascaridiasis evoke polar Th-1 and Th-2 host immune responses, respectively. A study to investigate the specific cytokine profile production by in vitro cultures of peripheral blood mononuclear cells from individuals living under precarious sanitary conditions in a highly endemic area for the parasites Toxoplasma gondii and Ascaris lumbricoides was conducted. High levels of both IFN-γ (Th-1) and IL-13 (Th-2) were observed in groups of co-infected individuals presenting toxoplasmic ocular lesions. Significantly lower IL-10 and TGF-β levels were produced by co-infected individuals in comparison with groups of individuals not infected with A. lumbricoides and either positive or negative for T. gondii living under good sanitary conditions (control groups). The possible influence of co-parasitism on the clinical presentation of ocular toxoplasmosis is discussed.*

**Key words:** *Toxoplasma gondii* - *Ascaris lumbricoides* - uveitis - retinochoroiditis - environmental contamination - cytokines

Concurrent parasitic infections are common among individuals living under poor sanitary conditions in developing countries. It has been suggested that helminthic infections can influence health both negatively, i.e., by worsening protective responses against *Mycobacterium tuberculosis* in HIV-infected individuals (Bentwich et al. 1999), and positively, by improving the adaptive immune response against inflammatory diseases (Renz et al. 2006, Weinstock & Elliot 2008).

Toxoplasmosis is generally asymptomatic in immunocompetent subjects. It may be acquired congenitally or at any time in life (Remington et al. 2006). Ocular toxoplasmosis is one of the main clinical manifestations in immunocompetent individuals and is considered a major cause of visual impairment (Holland 2003, 2004). The disease may be the result of recurring manifestations of congenital as well as acquired infections (Silveira et al. 1988, Holland et al. 1999). Many factors may influence the outcome of the disease, such as duration and frequency of exposure, route of infection, parasitic load, immunological factors and unidentified cofactors (McLeod et al. 1996, Garweg & Candolfi 2009).

Type 1 cytokines are of fundamental importance in the immune response to *Toxoplasma gondii* in competent hosts (Denkers & Gazzinelli 1998). *Ascaris lumbricoides* infections, as with other gastrointestinal helminthic infections, are characterized by eosinophilia, mast cell hyperplasia and elevated levels of circulating IgE. In endemic areas for ascaridiasis, helminth infections were associated with a highly polarized type 2 cytokine response (Cooper et al. 2000).

The determination of the prevalence of toxoplasmic ocular lesions in areas highly endemic for *T. gondii* constitutes a challenge in practice. This prevalence may diverge in areas of high serum prevalence. In general, for epidemiological studies, subjective criteria for the classification of retinal lesions are adopted and lesions are often categorized in terms of the probability of being caused by *T. gondii* infection (Glanser et al. 1992).

The present study was undertaken to investigate the immune response parameters of ex-vivo phenotypic profiles, specific cellular proliferation and cytokine production by peripheral blood mononuclear cells (PBMCs) from individuals living in endemic areas for *T. gondii* and *A. lumbricoides*, and their possible relationship with the occurrence of diversified clinical sets of toxoplasmic retinochoroidal scars. The data obtained open new perspectives for the investigation of parasite-specific immunomodulators, with the potential to influence the outcome of toxoplasmosis and ascaridiasis.

**PATIENTS, MATERIAL AND METHODS**

**Study population** - A total of 88 individuals participated in this study. They were identified in a survey of the prevalence and risk factors for *T. gondii* infection in Campos dos Goytacazes, a city located in Northern of
the state of Rio de Janeiro, with approximately 400,000 inhabitants and marked by economic and social divides (Bahia-Oliveira et al. 2003).

The following key variable definitions were adopted for the study population: (i) their sanitary conditions, (ii) the presence or absence of *T. gondii* and *A. lumbricoides* infections, and (iii) the presence or absence of retinal/retinochoroidal scars. Sanitary conditions were defined as poor/precarious if neither residential sewage facilities nor municipally-treated water was available and if the monthly income of the household was less than US$150. The *T. gondii* age-adjusted seroprevalence for this group was 84%. Sanitary conditions were defined as good if the monthly income of the household was more than US$500/month and if treated water and sewage facilities were available. The *T. gondii* age-adjusted seroprevalence for this group was 23% (Bahia-Oliveira et al. 2003).

All of the 88 individuals were screened for the presence of helminth eggs (Hoffman et al. 1934). Anti-helminth treatment (Mebendazol or Albendazol) was provided to all infected individuals. All the individuals living under precarious sanitary conditions (PSC) tested positive for *A. lumbricoides* and, of these individuals, some who were *T. gondii*-infected exhibited retinal/retinochoroidal lesion scars.

Individuals living in the same geographical area under good sanitary conditions (GSC), all of whom were negative for *A. lumbricoides*, comprised the control groups for this study. The control group individuals tested either negative or positive for toxoplasmosis; however, none of the positive ones presented retinal disorders. All groups were age and sex-matched and none were under drug therapy. None of the persons included had active inflammatory retinal lesions. No individual presented with clinical signs of malnutrition (WHO 1995).

The study protocol complied with the Helsinki Declaration and was approved by the Ethical Committee of Fundação Oswaldo Cruz-Fiocruz, Brazil, and the National Ethical Committee (CONEP 013/2007). Written informed consent was obtained from patients according to the guidelines of the Ethical Committee of Fiocruz.

**Classification of the retinal/retinochoroidal scars** - All the individuals presenting lesions were *T. gondii*-seropositive. The posterior retinal/retinochoroidal uveitis consistent with healed lesions was classified as either class A, B or C according to their morphologic aspects, observed with a 20 D or 78 D lens and an indirect ophthalmoscope (Bahia-Oliveira et al. 2001, Oréfice & Bahia-Oliveira 2005). The lesions were photographed (Topcon TRC-50X Retinal Fundus or digital ZEISS VISUCAM LITE Fundus). On average, each patient was examined by at least three ophthalmologists and a consensus was reached prior to lesion classification. The retinal/retinochoroidal scar size and localization were not considered as criteria in this classification; however, class C lesions were generally smaller than classes A and B. Class A lesions present well-marked boundaries, usually surrounded by a pigmented halo and extensive destruction of the retina and choroid. Class B lesions are characterized by a surrounding hypopigmented halo and a smaller degree of tissue destruction in comparison to class A. Class C lesions are basically areas of retinal pigmentary epithelium hyperplasia or atrophy with a smaller degree of tissue destruction compared to class A and B lesions, both of which fulfill all the morphological criteria of probably being caused by *T. gondii* infection. Because of the low degree of retinal destruction, class C lesions can be morphologically considered as a class of lesions of uncertain etiology.

Individuals presenting ocular lesions were examined twice, at the time of the survey and at the time this study was conducted (12-18 months after the survey). No difference was observed in any aspect of the lesions during this period.

**Antigens** - Antigenic preparations from *T. gondii* soluble tachyzoites antigen (STAg) were obtained from fibroblast cultures prepared as described previously (Gazzinelli et al. 1991) and used at a final concentration of 2 µg/mL. *A. lumbricoides* adult worms obtained from human faeces were used to prepare (ASC) antigen, derived from the whole body of the worm. Briefly, for ASC preparation, live worms from human faeces were washed several times with saline mixed with an equal volume of borate-buffered saline (BBS) pH 8.0 and homogenized in a Potter. After centrifugation at 12,000 g for 1 h, the precipitate was resuspended in BBS and stirred overnight at 4°C. The suspension was centrifuged again for 2 h at 12,000 g and the supernatant was dialysed against phosphate buffered saline (0.15 M, pH 7.2), filtered through 0.45 µm pore membranes for sterilization and used at a final concentration of 70 µg/mL (Soares et al. 1987). Staphylococcal enterotoxin B, SEB (SIGMA, St. Louis, USA) was used at a 0.25 µg/mL final concentration.

**Cell preparation and proliferation assay** - PBMCs were isolated by Ficoll-diatrizoate density gradient centrifugation (LSM, Organon Teknica, Charleston, SC, USA), as described previously (Gazzinelli et al. 1983). The culture medium consisted of 90.4% RPMI 1640, 16.1% L-glutamine, 3% of an antibiotic-antimycotic mixture stock (10,000 U penicillin, 5 µg streptomycin, 25µg fungizone per mL, GIBCO) and 5% heat-inactivated normal human serum AB, Rh+ (Sigma). For cytokine analysis, supernatants were collected from cultures of 1 x 106 cells per well in flat-bottom 24-well tissue plates. For the cell proliferation assay, 2.5 x 104 cells were cultured in flat-bottom 96-well tissue plates. Both types of plate cultures were maintained at 37°C in an atmosphere of 5% CO2 for six days.

Cells were exposed to 0.5 µCi of tritiated thymidine (methyl-3H thymidine, Amersham Life Science, Buckinghamshire, UK) per culture (specific activity 2.0 Ci/mM) for the last 6 h of culture and processed for scintillation counting. The data were calculated as mean counts per minute (CPM) of triplicate cultures and the results were expressed as experimental CPM - control (unstimulated) CPM (CPM = E-C).

**Flow cytometry analysis** - Ex vivo PBMCs were analyzed by cellular phenotypic analysis using two-colour flow cytometry in a Coulter EPICS® ELITE flow cytometer (Miami, FL, USA) for the following cell-surface markers: CD2, CD3, Pan γδ, CD4, CD8, CD16 and
CD19. A minimum of 3,000 gated (lymphocytes) cells were acquired. Monoclonal antibodies were purchased from Coulter Clone® (Miami, FL, USA), except for PE-conjugated antibodies against CD16 and Pan γδ (Immuno-tech-Coulter, Miami, USA).

Cytokine ELISA - Cytokine measurements were carried out by sandwich ELISAs using supernatants collected on different days according to previous kinetics studies performed in our laboratory (unpublished observations). One culture well was tested for each day supernatants were collected. Antibodies and standard cytokines were purchased from Pharmingen (San Diego, CA, USA) or R & D systems (Minneapolis, MN, USA). IL-13, TNF-α and TGF-β were measured in supernatants collected on the first day of culture, where the lower sensitivity cut-offs were 3.1 pg/mL, 5 pg/mL and 9 pg/mL, respectively. IL-5 and IL-6 were measured in supernatants collected on the third day of culture, where the lower cut-offs of sensitivity were 31.2 pg/mL and 0.6 pg/mL, respectively. IFN-γ, IL-12 and IL-10 were measured in supernatant collected on day six of culture, where the lower cut-offs of sensitivity were 15 pg/mL, 7 pg/mL and 6.3 pg/mL, respectively.

Avidity of anti- T. gondii IgG and anti- T. gondii IgM antibodies - The levels of T. gondii specific IgM and IgG avidity were measured in the sera of all anti- T. gondii IgG positive patients using the BioMerieux VIDAS Kit (BIOMERIUX, Lyon, France) according to the manufacturer’s recommendations.

Statistical analysis - Phenotypic analysis results, scintillation count values for proliferation assays, and cytokine measurements were log transformed before performing statistical analyses. Comparisons between the groups were made by Student t test, and \( p \leq 0.05 \) was considered as statistically significant with a 95% confidence interval.

RESULTS

Individuals were clustered in seven groups (Table) according to three parameters: (i) sanitary conditions, whether from areas of PSC or GSC; (ii) serology for T. gondii; and (iii) the presence (class A, B or C) or absence of scars of retinal/retinochoroidal lesions. Representative fundoscopic pictures of class A, class B and class C retinal/retinochoroidal lesion scars are shown in Fig. 1. T. gondii-positive sera samples were tested for the IgM and IgG avidity index. All of the values were either lower or higher, respectively, than the cut-off values considered for recent (less than 4 months) infections (data not shown), indicating that the individuals were chronically infected. With the exception of group 3 (with class A ocular lesions), all T. gondii-seropositive groups had significantly higher levels of cellular proliferation against T. gondii STAg in comparison with seronegative groups (Table). The levels of cellular proliferation induced by ASC antigens did not differ across the groups. Additionally, these levels were very low, sometimes similar to those of cultures without antigenic stimulation. Control groups living under GSC, which were negative for A. lumbricoides, presented similar levels of cellular proliferation as those observed in the infected group living under PSC when their PBMCs were stimulated with ASC antigens (Table).

The mean level of γδ T cells was higher in group 5 (patients with ocular lesions, class C) when compared with all the other groups, although the difference was not statistically significant. The levels of cells expressing CD2/CD16 (NK cells) was significantly higher in group 2 (patients living under PSC without ocular le-

### TABLE

<table>
<thead>
<tr>
<th>Groups</th>
<th>Toxoplasmosis serology/ocular lesion</th>
<th>Study group</th>
<th>n</th>
<th>Mean Age (SE)</th>
<th>NK cells % (SE)</th>
<th>γδ T cells % (SE)</th>
<th>Antigenic stimulation STAg ASC</th>
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<td>5.89 (1.19)</td>
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Subjects belonging to groups 1-5 presented infected with A. lumbricoides; subjects belonging to groups 6 and 7 were negative for A. lumbricoides. a: (NL) T. gondii-seropositive individuals without ocular lesions; b: d: the retinochoroidal scars lesions categorized respectively as class A, B, or C; e: precarious sanitary conditions (PSC) areas, all of the patients living in this area are infected with A. lumbricoides; f: good sanitary conditions areas(GSC); g: significant difference (p < 0.05) when compared to non-stimulated cultures; h: significant difference (p < 0.05) when compared to SN groups; i: significant difference (p < 0.05) when compared to SN groups; j: significant difference when compared to groups 1 (p < 0.05), 3 (p < 0.01), 6 and 7 (p < 0.001); STAg: soluble tachyzoites antigen. Asterisk: Δ cpm refers to values of cellular proliferation stimulated with antigens discounted from the values of cellular proliferation of the respective control cultures (not stimulated with antigens).
sions) in comparison with all the other groups (Table). The CD4/CD8 ratio was not different between the studied groups (not shown).

The following cytokines were evaluated in PBMC supernatant cultures: IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, TGF-β and TNF-α. Supernatants were collected on the day of the peak of each cytokine production as described in material and methods. Most of the cytokines had a peak-of-production day, except for IL-5, IL-6 and IL-10, which were continuously produced throughout the culture period (data not shown).

There were no significant differences between groups regarding the production of IL-5, IL-6 and IL-12 to SEB, parasitic antigens or media alone (data not shown). The levels of IL-5 in cultures stimulated with STAg and ASC antigens were generally lower than in control cultures not stimulated with antigens (data not shown).

The levels of IL-10 and TGF-β detected in cultures stimulated with SEB, STAg and ASC are shown in Fig. 2A, B, respectively. No difference was observed between groups living under PSC, either with or without ocular lesions. However, patients living under GSC produced both cytokines at elevated levels (p < 0.05) in comparison with groups living under PSC in response to both antigens and in control cultures (not stimulated with antigens). Nevertheless, no differences were observed in cultures stimulated with SEB.

When stimulated with STAg, the mean levels of IL-13 production in groups with ocular disease (class A, B and C lesions) were significantly higher than those observed for groups either negative or positive for toxoplasmosis and without lesions, as shown in Fig. 3A. The highest levels of IL-13 were secreted by PBMCs of patients belonging to the class C lesion group by stimulation with either STAg or ASC. All of the groups living under PSC secreted significantly higher levels of IL-13 under stimulation with ASC antigens in comparison with groups living under GSC (Fig. 3A); stimulation with SEB yielded no difference between the groups (data not shown). There was no significant difference between the levels of IL-13 secreted by PBMCs from toxo-seropositive and seronegative patients living under GSC (Fig. 3A) when stimulated with any antigenic preparation or SEB (data not shown).

The levels of TNF-α were low for the majority of the groups in response to both antigens (Fig. 3B). However, they were significantly higher in supernatants of cultures stimulated with STAg from subjects of group 2 (T. gondii).
**DISCUSSION**

Beyond parasite control, a better understanding of parasite immunomodulation can ultimately contribute to the development of medical applications. Many examples illustrate this potential of parasites’ molecules or extracts employed in experimental models. For example, the filarial nematode secreted product ES-62 has been shown to have both prophylactic and therapeutic efficacy in a murine model of arthritis (Harnett et al. 2004). Recently, anti-inflammatory ES-62 activity was found in synovial tissues from patients with rheumatoid arthritis (Harnett et al. 2008).

*T. gondii* and *A. lumbricoides* are both parasites that infect hosts orally; however, they elicit polar type I or type II host responses, respectively. Since both parasites are endemic in tropical areas, it is likely that co-infections with these organisms have been common throughout human evolution. If this is the case, then the host immune response mounted against both parasites may have adapted to permit such co-parasitism.

The epidemiological features of toxoplasmosis and ascariasis in the studied area must be considered in order to better understand the possible influence of each parasite on the outcome of infection by the other. Drinking water has been determined as the main risk factor for *T. gondii* infection for individuals living under PSC.
T. gondii oocysts have been detected in consumable well water in the dwellings of individuals who took part in this study (unpublished observations). Considering that T. gondii oocysts are viable in water for long periods (Dubey 1998), we argue that, for the studied population, exposure to T. gondii antigens probably occurs very frequently and very early in life, according to the prevalence curve for the poor population involved in the present study (Bahia-Oliveira et al. 2003). Exposure to A. lumbricoides is also likely to occur very frequently, since all the patients living under PSC were infected with the helminth and the prevalence of this infection is no lower than 90% in areas of dos Campos dos Goytacazes with poor sanitation (unpublished observations).

Late development of ocular toxoplasmic lesions can occur in cases of either congenital or acquired infection (Cooper et al. 2000). Because the present experimental setting did not address whether T. gondii infections were acquired or congenital, we will consider the profiles of immunologic parameters in light of the diversity of the clinical presentations of retinal/retinochoroidal scars found in T. gondii-seropositive patients co-infected with A. lumbricoides.

Generally, in endemic areas for A. lumbricoides, it has been shown that older individuals acquire resistance to the helminthic infection, which is associated with the cumulative capacity of older persons to produce anti-helminth IgE (Woolhouse et al. 1991). In the studied area, however, we did not observe this tendency. The in vitro immune response against T. gondii and A. lumbricoides antigens of co-infected individuals was marked by secretion of low levels of IL-10, IL-4, IL-5 and TGF-β as well as by low levels of specific IgE against A. lumbricoides (data not shown). This aspect would favour the adaptation of the helminth to the host, since the specific protective immune response for that parasite is down-regulated. The relatively low impact of toxoplasmic ocular lesions (10-12%) is in contrast with the high serum prevalence of toxoplasmosis, which is 84% (adjusted for age) for persons of low socioeconomic income living in areas of precarious/poor sanitation in the present study. The T. gondii-specific immune response of patients presenting ocular lesions is marked by secretion of high levels of IFN-γ and IL-13. Both cytokines might concomitantly and respectively limit the parasite’s growth and control the inflammatory response within the eye, which would result in a better adaptation of T. gondii to the host. The higher levels of IL-13 secreted by PBMCs from co-infected individuals in comparison with the levels of this cytokine secreted by those infected only with T. gondii corroborate the idea that A. lumbricoides infection would favour the adaptation of T. gondii to the host, since the impact of eye infection would be lower (producing a less aggressive inflammatory response within the eye) and improve the chances of parasitism success. These data are in agreement with reports demonstrating that, in rats with endotoxin-induced uveitis, the systemic or intraocular injection of IL-13 significantly inhibited the production of pro-inflammatory cytokines and resulted in less intense ocular inflammation without down-regulating the levels of local IFN-γ (Lemaitre et al. 2001). IL-13 has been considered an important cytokine for resistance to experimental intestinal nematode infections (Bancroft et al. 1998). However, the co-infected individuals did not secrete high levels of IL-13 under A. lumbricoides antigenic stimulation and its role in the helminthic infections for the studied population should be further investigated.

Aspects of the immune response of the studied individuals suggest that co-parasitism prompts the immune system to mount specific responses that differ from those observed in single-parasite infections. Considering the epidemiological and immunological aspects related to the studied area, we propose that (i) the co-evolution might have resulted in a better adaptation for both parasites to their hosts and (ii) this adaptation was driven by products of parasites being able to modulate the host immune response.

Stoicov et al. (2004) demonstrated that T. gondii infection in a host infected with Helicobacter felis alters the natural outcome of T. gondii infection, allowing uncontrolled tachyzoite replication and severe organ damage and demonstrating the profound interactions of the immune response to unrelated organisms. Recently, Wagner et al. (2008), using a mouse model of birch pollen allergy, investigated whether infection with T. gondii influences allergic immune responses to birch pollen. It was demonstrated that T. gondii exhibits strong immunomodulating properties that lead to the prevention of Th-2 allergic immune responses. Curiously - and in contrast to our data - the infection was associated with enhanced TGF-β, IL-10 and Foxp3 mRNA, probably involved in suppression of the allergic immune response.

Anecdotal reports by local ophthalmologists support the view that recurrent T. gondii lesion activation rarely occurs in the studied area. Furthermore, no episode of lesion reactivation was reported during the 12-18 months when this study was conducted. If helminthic infections can improve the adaptive immune response against inflammatory diseases, then the host immune response to A. lumbricoides, which led to high levels of IL-13 production, may have benefited patients since it could potentially down-modulate pro-inflammatory cytokines without down-modulating IFN-γ secretion, a cytokine required for T. gondii elimination (Suzuki et al. 1988).

Higher levels of IFN-γ, as well as IL-13, were observed in patients presenting class C lesions, which are characterized by less severe retinal damage, than in patients with class A and class B lesions. The high levels of IL-13 regulating the inflammatory process in the presence of high levels of IFN-γ controlling T. gondii replication could, in principle, promote parasite growth control with minor tissue damage.

Because IFN-γ and IL-13 are Th-1 and Th-2 cytokines, respectively, it was expected that their levels would correlate inversely. Surprisingly, as estimated by Spearman’s correlation, the secretion of these cytokines followed a positive correlation pattern instead, which, however, was not statistically significant (data not shown). We speculate that the positive correlation observed between IFN-γ and IL-13 indicates that the cytokines do not inhibit the secretion of each other; rather, they might
share a synergistic relationship. Evidence of synergism between IFN-γ and IL-13 in the eye has been described in the experimental model of endotoxin-induced uveitis in rats where it was shown that IL-13 promoted a decreased expression of TNF-α, IL-1β, IL-6, monocyte chemoattractant protein - MCP-1 - and macrophage inflammatory protein - MIP-2 - as measured by mRNA levels in the iris-ciliary body and the retina while IFN-γ was up-regulated in the iris-ciliary body (Lemaître et al. 2001). That study thereby showed that the role of IL-13 in protecting against the damage of a strong inflammatory response in the eye.

Supporting this hypothesis are the data published by Yamamoto et al. (2000), showing that in the South of Brazil (living in the city of Erechim), where toxoplasmosis is highly prevalent and lesion reactivation is often observed (Silveira et al. 2001), the levels of IL-4 and IFN-γ secretion were low in PBMC cultures from patients with eye lesions (Yamamoto et al. 2000). These differences might reflect variations in the clinical presentation of ocular diseases in both endemic areas of Brazil. Although toxoplasmosis is equally and highly prevalent in both areas (~80%), the prevalence of retinochoroiditis in Campos dos Goytacazes is much lower (~12%) than in Erechim (~25%). High genetic diversity of *T. gondii* isolates from Brazil has been described (Dubey et al. 2008) and the allelic diversity of parasite proteins, important in interactions with host cells, might partly explain the differences in the profiles of host immune response as demonstrated by Saeij et al. (2007), who described ROP 16 interfering with STAT 3 and STAT 6 host transcription factors.

We did not quantify the parasitic helminthic burden in the present study and we therefore cannot infer its effect on the clinical presentation of the ocular lesions. Furthermore, because we have not evaluated the presence of other intestinal parasites, we cannot rule out the role of the response against other organisms that could elicit components of Th-2 or Th-1 immune responses.

The low degree of retinal destruction in group 3 (individuals with class C lesions) was also associated with higher levels of circulating γδ T-cells compared to all the other groups studied. Elevated levels of γδ T-cells have been associated with acute *T. gondii* infection (De Paoli et al. 1992). These results led us to investigate the levels of IgM and IgG avidity in their sera to estimate the duration of infection. Despite the cellular evidences of recent infections, the IgM and IgG avidity data did not confirm recent infection of less than four months. Therefore, it is not possible to appraise with much certainty whether class C lesions are more recently acquired lesions that will evolve to class A or B destructive lesions or whether they are an entity of lesions that are not normally registered, because they do not produce important symptoms. However, during the period of 12-18 months of this study, the registered C lesions did not evolve to class A or B, suggesting that they may constitute a class of toxoplasmic lesions that are not commonly detected, probably because of their low clinical impact or lack thereof, or because their evolution to class A or B is a rare and/or slow event.

The significance of the high levels of TNF-α secreted by PBMCs from individuals with antibodies to *T. gondii* living under PSC and presenting no retinal scar tissue, as well as the presence of elevated levels of NK cells, remains to be further investigated in the context of protection against the development of toxoplasmic retinochoroiditis.

Although it is unclear whether the participating individuals had been infected congenitally or postnatally, we reason that at least some patients presenting class A lesions had been infected congenitally. Three parameters support this hypothesis: (i) the high prevalence of congenital toxoplasmosis in Campos dos Goytacazes (Bahia-Oliveira et al. 2001); (ii) the morphological aspects and the extent of tissue damage of class A lesions, since severe retinal/retinochoroidal damage is often observed in congenitally infected patients; and (iii) the fact that those individuals exhibited lower levels of PBMC proliferation under *T. gondii* stimulation in comparison with groups presenting no lesions or with other classes of lesions. In fact, the low capacity of PBMC proliferation in response to *T. gondii* in vitro stimulation has been associated with congenital infection, even in adults (Yamamoto et al. 2000). The observed low levels of PBMC proliferation in response to ASC stimulation is probably due to the inhibition of cellular proliferation that has been described for the murine model immunized with *Ascaris suum* antigens. High molecular weight components were responsible for its suppressive effect upon Th-1 and Th-2-dependent immune responses to unrelated antigens (Faquim-Mauro & Macedo 1998).

Finally, the present study provides evidence for the importance of considering the natural conditions of antigenic exposure to commonly occurring parasites when investigating the role of immunologic parameters and adaptive immune responses to these parasites on disease outcomes.

The value of classifying toxoplasmic ocular lesions in terms of the degree of retinochoroidal destruction is highlighted by the data presented here. Follow-up of individuals presenting class C lesions, in this and other geographical areas, will be needed to improve our understanding and could potentially contribute to the development of immune therapy for ocular toxoplasmosis.

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